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Effects of formaldehyde exposure on anxiety-like and depression-like behavior, cognition, central levels of glucocorticoid receptor and tyrosine hydroxylase in mice

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HIGHLIGHTS

- Inhalation of 2 ppm formaldehyde reduced the body weight.
- This treatment increased levels of depression-like behavior.
- This treatment impaired novel object recognition.
- This treatment lowered the levels of glucocorticoid receptors in the hippocampus.
- This treatment reduced levels of brain tyrosine hydroxylase.

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ABSTRACT

Formaldehyde exposure is toxic to the brains of mammals, but the mechanism remains unclear. We investigated the effects of inhaled formaldehyde on anxiety, depression, cognitive capacity and central levels of glucocorticoid receptor and tyrosine hydroxylase in mice. After exposure to 0, 1 or 2 ppm gaseous formaldehyde for one week, we measured anxiety-like behavior using open field and elevated plus-maze tests, depression-like behavior using a forced swimming test, learning and memory using novel object recognition tests, levels of glucocorticoid receptors in the hippocampus and tyrosine hydroxylase in the Arc, MPOA, ZI and VTA using immuhistochemistry. We found that inhalation of 1 ppm formaldehyde reduced levels of anxiety-like behavior. Inhalation of 2 ppm formaldehyde reduced body weight, but increased levels of depression-like behavior, impaired novel object recognition, and lowered the numbers of glucocorticoid receptor immonureactive neurons in the hippocampus and tyrosine hydroxylase immonureactive neurons in the ventral tegmental area and the zona incerta, medial preoptic area. Different concentrations of gaseous formaldehyde result in different effects on anxiety, depression-like behavior and cognition ability which may be associated with alterations in hippocampal glucocorticoid receptors and brain tyrosine hydroxylase levels.

1. Introduction

Formaldehyde (FA) is a cheap, volatile organic compound with active chemical properties wildly used in construction materials (Harrison, 2002) such as man-made plate and paint. FA is a common indoor air pollutant and can attain relatively high concentrations in homes and buildings. This compound easily passes through the blood–brain barrier, eventually accumulates in the human body (Nazaroff and Singer, 2004), and has the potential to affect neuroglial and nerve cells in the brain (Malek et al., 2003a) and alter emotional or cognitional behaviors; however, potential mechanisms for these effects are poorly understood.

Inhalative formaldehyde exposure or injection of formaldehyde at pathological levels (0.5 mM) directly into the hippocampus of rodents induces a deficit in spatial learning and memory in a Morris water maze test (Pitten et al., 2000; Malek et al., 2002, 2003b; Lu et al., 2008; Tong et al., 2015). However, unlike the Morris water maze test which is often used to investigate spatial learning

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Abbreviations

Arc	arcuate nucleus
DA	dopamine
DI	discrimination index
EPM	elevated plus-maze test
FA	formaldehyde
FST	forced swimming test
GR	glucocorticoid receptor
GR-IRs	GR immunoreactive neurons
HPA	hypothalamo-pituitary-adrenal axis
MPOA	medial preoptic area
NOR	novel object recognition
OFT	open-field test
ppm	parts per million
TH	Tyrosine hydroxylase
TH-IRs	TH immunoreactive neurons
VTA	ventral tegmental area
ZI	zona incerta

and memory (Malek et al., 2003b; Pitten et al., 2000), the novel object recognition (NOR) task for rodents is a non-spatial, non-aversive memory test (Reynolds and Wright, 1979) used to test the formation of memories regarding the location and identity of objects (Lynch et al., 2011). Unlike the Morris water maze which tests the efficiency to find a hidden platform and indicates cognitive efficiency, the novel object recognition test measures cognitive capability (Lynch et al., 2011). As a result, same drug treatments often induce different effect in Morris water maze and novel object recognition tests (Milić et al., 2013). Although the effects of FA on spatial learning and memory have been profoundly investigated using Morris water mazes, whether formaldehyde exposure affects novel object recognition and neural mechanism for this remains unclear.

The hippocampus is a limbic area of the central nervous system involved in emotion, memory and learning. It is known to possess the greatest levels of adrenocorticosteroid receptor binding and mRNA expression (Reul and deKloet, 1985; Aronsson et al., 1988; Reul et al., 1989) which more likely underlie the deleterious effects of glucocorticoids on learning and memory and long-term potentiation (Diamond and Rose, 1994; Bodnoff et al., 1995). Chemical exposure such as FA exposure can activate the hypothalamo-pituitary-adrenal gland (HPA) axis and subsequently increase levels of glucocorticoids (Dallman et al., 2003; Makino et al., 2002; Sorg et al., 2001a) that regulate the HPA response via negative feedback of GRs binding in the hippocampus (Raone et al., 2007). Repeated stress exposure and long-term glucocorticoid treatment downregulate levels of GRs in different brain areas, particularly the hippocampus (Huot et al., 2004; Kitraki et al., 2004). Here, we predict that repeated FA exposure possibly reduces levels of GRs in the hippocampus. Low levels of GRs in the hippocampus induce less negative feedback to regulate levels of CORT (Kitraki et al., 2004), subsequently increase levels of CORT and lead to higher levels of anxiety and depression. In order to test this hypothesis we used open-field tests (OFT), elevated plus-maze tests (EPM) and forced swimming tests (FST) to assess levels of anxiety- and depression-like behaviors following repeated FA exposure.

Acute FA exposure at 5 ppm results in decreases motor activity and increased levels of dopamine (DA) together with its enzymatic metabolites in the hypothalamus of rats (Boja et al., 1985). Furthermore, two weeks of inhalative FA treatment (13.5 ± 1.5 ppm) enhances aggressive behavior and increases DA in the frontal cortex synaptosome (Liu et al., 2009). Thus, changes in behavior induced by FA exposure may also be associated with alteration in DA levels because DA plays a role in the control motor activity and emotional behavior (Gainetdinov et al., 1999; Rodgers et al., 1994; Zhuang et al., 1999; Zhou and Palmiter, 1995). Tyrosine hydroxylase (TH), a rate limiting enzyme for DA synthesis, as an indicator of DA production, is found in many areas of the rat brain. Here, we investigated whether gaseous FA exposure could alter TH expression as a way of exploring the mechanistic and underlying effects of FA exposure on emotional and cognitive behaviors.

Taken together, our aims are to investigate the effects of FA exposure on levels of anxiety, depression-like behavior and novel object recognition and to determine whether any effect on behaviors is associated with levels of hippocampal GR and TH expression in the brain.

2. Materials and methods

2.1. Animals and treatment procedure

Male Kunming mice (an outbred stock of Swiss albino mouse commonly used in toxicological and pharmaceutical research) (n = 45; 7 weeks old) were obtained from Xi'an Jiao tong University Laboratory Animal Center (Shaanxi, China). All animals were housed five per cage in standard transparent Makrolon cages (42 cm × 26 cm × 20 cm) and acclimated housing conditions for two weeks prior to experiments. Food and water were given ad libitum and the animals were kept at a constant temperature (21 ± 2 °C) and humidity (40 ± 5%) under a reversed light: dark 12:12 cycle (lights on at 2200 h). The maintenance and treatment of animals were in accordance with the Guidelines for the Care and Use of Laboratory Animals and our protocols were approved by the Animal Care and Use Committee of Shaanxi Normal University.

2.2. Gaseous formaldehyde exposure

The animals, weighing between 34 g and 37 g (9 weeks old) were randomly divided into three groups: 0, 1 and 2 ppm FA inhaled groups. Different concentrations of gaseous FA were generated by evaporation of formalin solution (37%, Xi'an Chemical Works, Xi'an, China) in static wood toxification chambers (54 cm × 54 cm × 54 cm) with three cages inside; each cage contained five mice during each inhalative FA treatment. The concentration of evaporated FA was monitored by a FA digital electrochemical analyzer (DM100-CH_2O, Nanjing Diandian Electronics Technology, Nanjing, China) to be around 1 ± 0.04 ppm and 2 ± 0.12 during the procedure.

When animals were exposed to gaseous FA they were affected as gas dissolved into their body fluids; this mode is similar to that in humans. The inhaled groups were exposed to different concentrations of gaseous FA from 1000 to 1200 h, 2 h per day for 7 d. During exposure, animals were not allowed to drink or eat. Gaseous FA concentrations generated from chamber emissions were measured three times per day for 7 d. The experimental results (0, 1 ± 0.04 and 2 ± 0.12 ppm) were very close to designed concentrations (0, 1 and 2 ppm). Gaseous FA levels from the chamber were steady and reliable.

2.3. Behavioral tests

After exposure to FA on the seventh day, subjects were returned to their original room and left without further disturbance. Two hours later animals were used for behavior tests (open-field test, elevated plus-maze test, forced swimming test and novel object recognition test). All behavioral tests were performed daily between 1400 and 1800 h and the test of least stress was first. The interval was 24 h between tests in order to avoid the effect of the

forced swimming test; the interval between novel object recognition test and forced swimming test was 2 d.

2.3.1. Open field test

Spontaneous motor activity and anxiety-like behavior were assessed in an open-field chamber consisting of a square arena (50 cm × 50 cm × 50 cm) made of gray glacial polyvinyl chloride, brightly and evenly illuminated by six 60 W lamps mounted 2 m above the arena. The area was divided into 16 quadrants (four central and 12 peripheral). Mice were individually placed into the center of the open-field and allowed to explore for 5 min. Time spent in the central and peripheral zones, total distance traveled during the experiment and the number of crossings between quadrants were recorded using a digital video camera and scored by Videomot2 (TSE Systems, Bad Homburg, Germany). The apparatus was cleaned using 70% ethanol after each animal.

2.3.2. Elevated plus-maze test

The EPM apparatus was constructed from gray glacial polyvinyl chloride and consisted of four arms (30 cm × 7 cm) connected to a central area (7 cm × 7 cm) elevated 1 m above the floor. Two arms were open and two were closed with 20 cm high walls. Mice were placed individually in the central area facing an open arm and were observed for 5 min. Total distance traveled during the experiment, number of crossings between closed and opened arms, as well as time spent in the open and closed arms were detected using the same equipment as in the open-field test above. The apparatus was cleaned using 70% ethanol after each animal.

2.3.3. Forced swimming test

The forced swimming test employed was essentially similar to that described elsewhere (Petit-Demouliere et al., 2005). Briefly, mice were dropped individually into glass cylinders (height: 25 cm; diameter:15 cm), containing 15 cm water, maintained at 23–25 °C, and remained there for 6 min. The cylinder was rinsed thoroughly and filled with clean water prior to testing each animal. Once the test began, the duration of immobility (the mouse floated in an upright position and made only small movements to keep its head above water) was recorded during the last 5 min of the 6-min testing period. Each animal was returned to the home cage immediately following the swim period, and was placed under a warming lamp for a total of 15 min. Behaviors were recorded by a digital video camera and scored later by an experimentally blind observer using Observer 5.0 (Noldus, the Netherlands).

2.3.4. Novel object recognition test

The novel object recognition test was performed following standard procedures as described and used in Mamiya et al. (2008). The test procedure consisted of three sessions: habituation, training and retention. Each mouse was individually habituated to the box (30 cm × 20 cm × 13 cm), with 10 min of exploration in the absence of objects for 2 d (habituation session). During the training session two identical objects were placed in the back corner of the box. The experimental mouse was then placed midway at the front of the box and the total time spent exploring the two objects was recorded for 5 min. During the retention session, animals were placed back into the same box 24 h after the training session, in which one of the familiar objects used during training was replaced with a novel object. The animals were then allowed to explore freely for 5 min and the time spent exploring each object was recorded. Throughout the experiments the objects were used in a counterbalanced manner in terms of their physical complexity and emotional neutrality. Discrimination between two objects was calculated using a discrimination index (DI), [DI = (novel object exploration time/total exploration time) − (familial object exploration time/total exploration time) × 100], which takes into account individual differences in total exploration time (Dix and Aggleton, 1999; Ennaceur and Delacour, 1988).

2.4. Immunohistochemistry

After the seventh day of FA inhalation, subjects were returned to their own cages and left without further disturbance. Twenty-four hours after behavioral tests, animals (n = 21) were then deeply anaesthetized and perfused with 0.1 M phosphate buffer solution (PBS, pH 7.4) and 4% paraformaldehyde in 0.1 M PBS. The brain was removed and placed in 4% paraformaldehyde overnight. Prior to dissection brains were immersed in 30% sucrose until saturated. Coronal sections (40 μm) were cut on a cryostat and consecutive sections collected in two vials containing 0.01 M PBS to enable two different immunohistochemical stainings. GR (sc-1004; Santa Cruz, California, USA) and TH (ab112, Abcam lid, Hong Kong, China) antibodies were rabbit polyclonal types. Floating sections were processed using the primary antibody and Streptavidin/Peroxidase methods (Bioss Company, Beijing, China). We incubated each vial per brain for 10 min with 3% H_2O_2, and then washed for 2 × 10 min with distilled water.We shrunk tissue in 0.01 PBS. Sections were preincubated for 60 min with normal goat serum (SP-0023) and incubated at 4 °C overnight in primary antibody solution (GR antibody, 1:100; TH antibody 1:150) diluted using antibody diluent (0.01 M PBS containing 20% bovine serum albumin and 1.7% Trition-X-100). The next day sections were washed 4 × 5 min with 0.01 M PBS and incubated for 90 min in a 37 °C water bath with biotinylated goat anti-rabbit antibody (SP-0023), followed by another round of 4 × 5 min 0.01 M PBS. After 150 min of incubation with SABC and 4 × 10 min washing with 0.01 M PBS, sections were stained with 3,3′-diaminobenzidine tetrahydrochloride (DAB) for visualization of immunoreactivity.

2.5. Measurement of immunoreactive neurons

An Olympus microscope was used to count stained nuclei. Slides were randomized and coded for microscopic analysis so that counters were blind to experimental treatment. The numbers of immunoreactive neurons were quantified by eye with the aid of a reticle placed in one ocular lens. For each brain nucleus, three representative sections from anterior to posterior anatomically matched between subjects were chosen and counted to minimize variability. Individual means were obtained by counting positive neurons bilaterally in three sections from each nucleus. Counts were performed separately for each hemisphere and results were averaged between hemispheres. Sections were chosen by correspondence to the reference atlas plate and not by the level or intensity of GR-IR and TH-IR labeling. GR-IR were quantified in CA1 (Interaural = 1.50 mm; Bregma = 2.30 mm), TH-IR were quantified in Arc (Interaural = 2.34 mm, Bregma = 1.46 mm), MPOA (Interaural = 3.34 mm, Bregma = 0.46 mm), ZI (Interaural = 2.86 mm; Bregma = 0.94 mm) and VTA (Interaural = 0.72 mm, Bregma = 3.08 mm), Around these positions, these regions are also counted. GR immunostaining was detected in the CA1 area of the hippocampus which is known as a crucial region in

Table 1
Effect of formaldehyde exposure on body weight.

	Control males	1 ppm group	2 ppm group
Body weight(g)			
Initial	35.7533 ± 0.5576	35.5733 ± 0.8074	35.7357 ± 0.4674
Final	37.5667 ± 0.5247	34.1000 ± 0.8439	32.0214 ± 0.4699*

Values are mean ± SEM.*Significant difference between groups ($p < 0.05$).

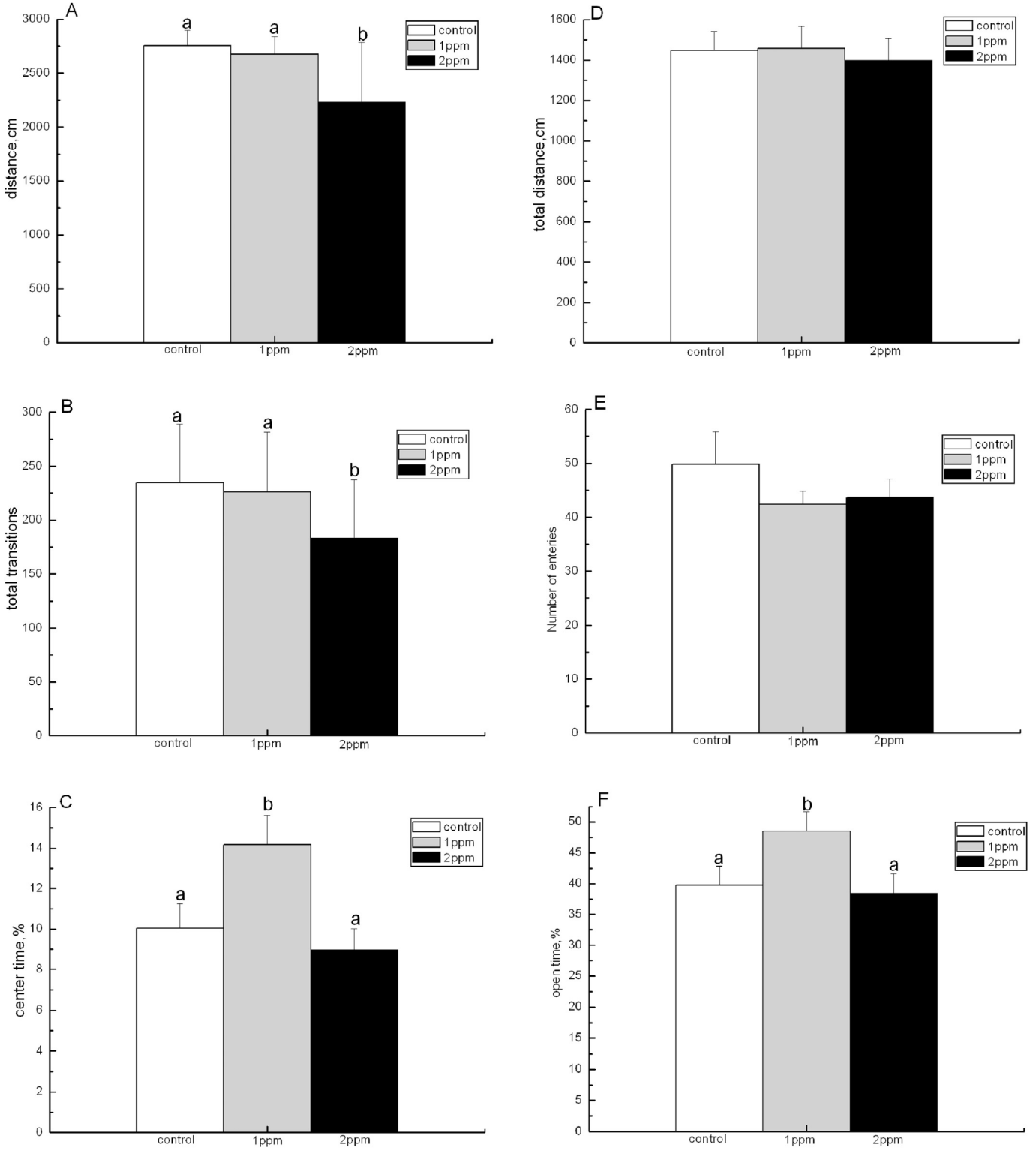

Fig. 1. Total distance moved (A), total number of transitions (B) and percentage of time in central area(C) in the open field test. Total distance moved (D), total enteries into open arms (E) and the percentage of time spent in open arms (F) in the elevated plus-maze test. Groups not sharing same letters are significantly different.

both the manifestation of depression-related behavior and integration of the stress response (McLaughlin et al., 2007). TH immunostaining was detected in the arcuate nucleus (Arc), ventral tegmental area (VTA) and the zona incerta (ZI), in which dopaminergic neurons are located in high densities (Baskerville and Douglas, 2010). Chosen sections were photographed with a Nikon (Tokyo, Japan) camera attached to an Olympus microscope.

2.6. Statistical analyses

All data were found to be normally distributed using a one-sample Kolmogorov–Smirnov test. Data from the open field test, elevated plus-maze test, forced swimming test, novel object recognition test, adult body weight, and numbers of GR-IR and TH-IR neurons were analyzed using one-way ANOVA with treatment

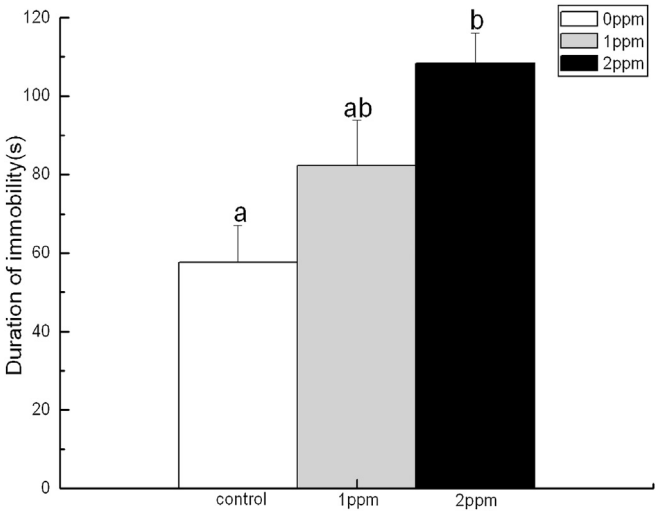

Fig. 2. Total immobility time in forced swimming test. Groups not sharing same letters are significantly different.

as the factor. Post hoc tests were conducted using Fisher's least-significant difference (LSD). Correlations between the open field test, elevated plus-maze test, forced swimming test, and data from novel object recognition and GR-IR and TH-IR were analyzed using Pearson correlations. All data are presented as mean ± standard error (SE) in figures and significance was set at $P < 0.05$. All statistical analyses were conducted using SPSS 10.0 (SPSS Inc., Chicago, USA).

3. Result

3.1. Changes in body weight

Body weight was measured before and after formaldehyde inhalation for 7 d. Formaldehyde inhalation of 2 ppm reduced body weight ($F_{(2, 42)} = 12.09$, $p = 0.000$); however body weight was not affected at 1 ppm FA inhalation (Table 1).

3.2. Behavioral change after inhalation formaldehyde

In the OFT, the three groups differed in the total distance traveled in the open field ($F_{(2, 42)} = 3.25$, $p = 0.050$; Fig. 1A), total transitions between quadrants ($F_{(2, 42)} = 3.31$, $p = 0.05$; Fig. 1B) and the time spent in the center of the open field ($F_{(2, 42)} = 4.78$, $p = 0.014$; Fig. 1C). Subjects exposed to 2 ppm FA also moved a shorter distance than the 1 ppm group ($p = 0.045$) and controls ($p = 0.025$). Individuals exposed to 2 ppm FA crossed less frequently compared to the 1 ppm group ($p = 0.044$) and controls ($p = 0.024$). Animals exposed to 1 ppm FA spent more time in the center of the open field than 2 ppm animals ($p = 0.006$) and controls ($p = 0.030$).

In the EPM, one way ANOVA showed that the percentage of time spent in the open arms was affected by FA exposure ($F_{(2, 42)} = 3.18$, $p = 0.049$). Post hoc tests demonstrated that animals exposed to 1 ppm FA spent more time in the open arms than the 2 ppm group ($p = 0.043$) and control mice ($p = 0.026$) (Fig. 1D–F).

In the FST, immobility time was affected by FA exposure ($F_{(2, 42)} = 5.15$, $p = 0.011$). Mice in the 2 ppm group spent more time immobile than controls ($p = 0.003$); there was no difference between 1 ppm animals and controls (Fig. 2).

In the NOR, males from the three groups spent equal amounts of time exploring either of the two objects in the training session (Fig. 3A), thus there was no biased exploratory preference among

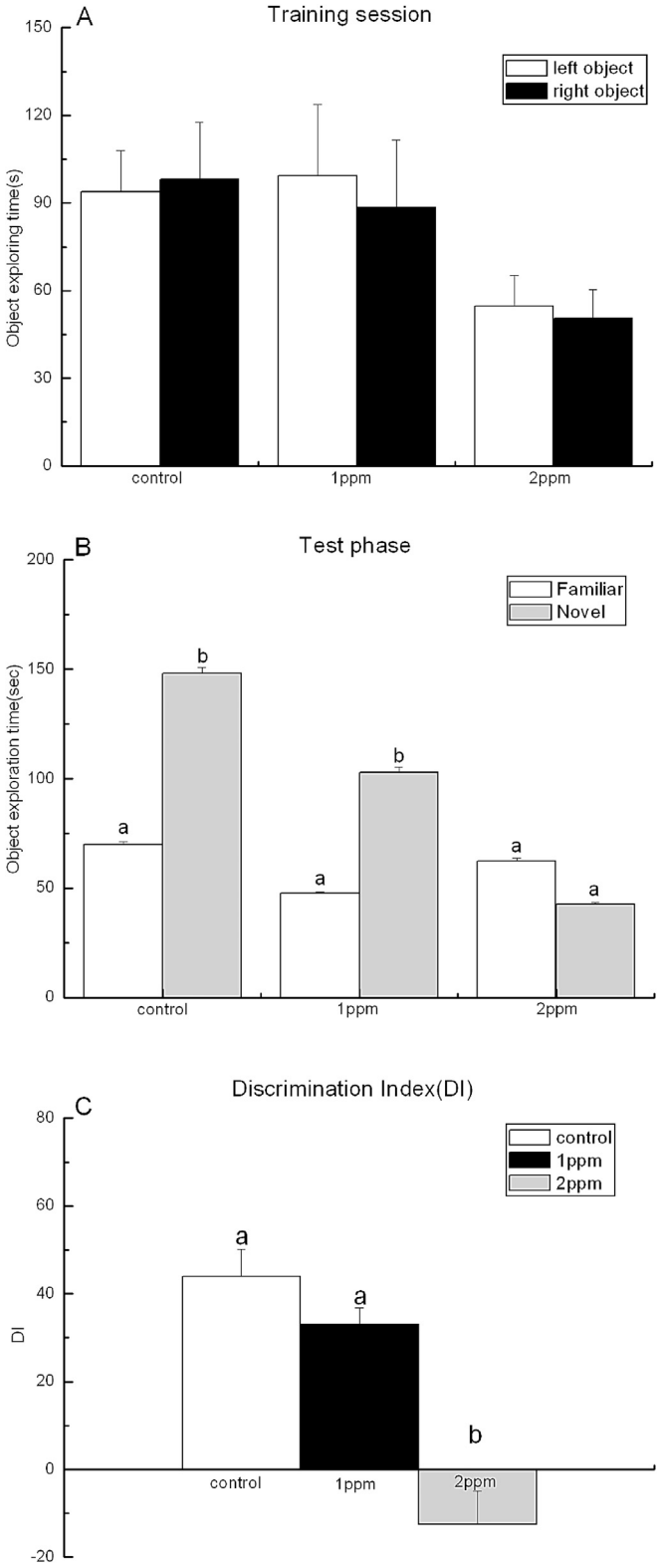

Fig. 3. Exploration time(s) of identical objects in training session (A), exploring time of the novel object in retention session (B) and discrimination index (C). Groups not sharing same letters are significantly different.

treatments. During the retention performance test, object exploration time for the novel object was higher in mice exposed to 1 ppm FA or controls ($p < 0.05$, Fig. 3B). We found that the DI in the 1 ppm group did not change compared with controls, but the DI in the 2 ppm exposed groups was reduced compared to control

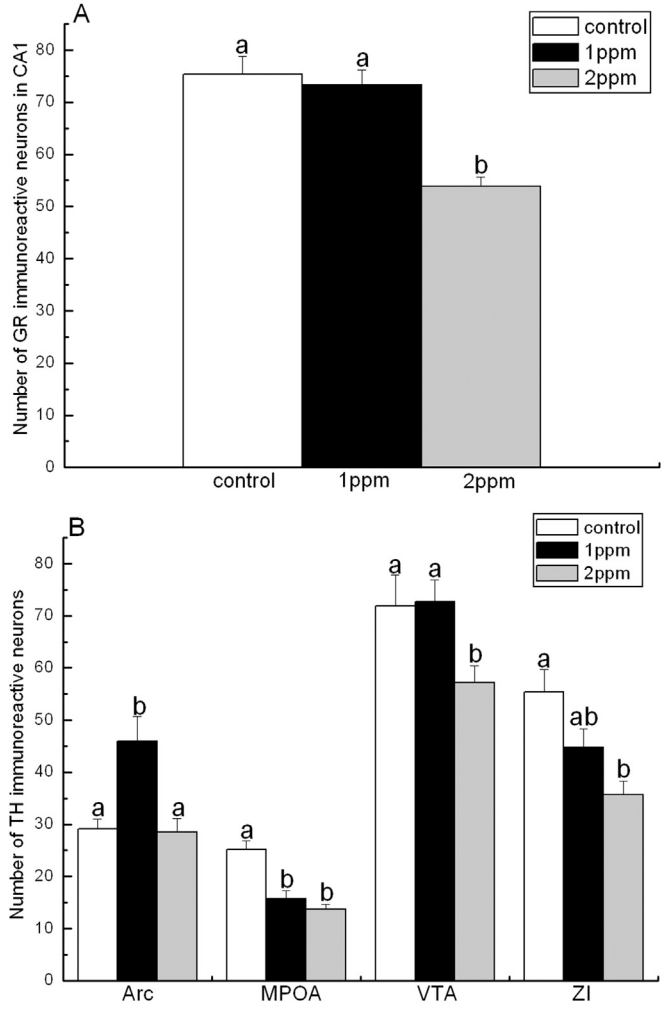

Fig. 4. Mean ± SEM number of GR-ir neurons in the hippocampus of mice across the three experimental groups (A). Mean ± SEM number of TH-ir neurons in the brain of mice across the three experimental groups (B). Groups not sharing same letters are significantly different.

and 1 ppm animals ($p < 0.05$, Fig. 3C). This indicates that mice exposed to 2 ppm FA could not discriminate a novel object from a familiar object.

3.3. GR-ir neurons in the hippocampus

One way ANOVA demonstrated that FA exposure affected the number of GR-IR neurons in the hippocampus ($F_{(2, 57)} = 18.05$, $p = 0.000$). Post hoc tests found that 2 ppm FA groups had fewer GR-IR neurons compared with the 1 ppm group ($p < 0.001$) and controls ($p < 0.001$). Exposure at 1 ppm FA had no effect (Fig. 4A and 5). Using Pearson correlation analysis we found a negative correlation between GR-IR and forced swimming test ($r = -0.347$, $p = 0.035$), and a positive correlation between GR-IR and novel object recognition ($r = 0.605$, $p = 0.001$).

3.4. TH–ir neurons

One way ANOVA revealed that the number of TH-IR neurons varied with FA exposure (Arcuate nucleus: $F_{(2, 52)} = 9.19$, $p = 0.000$; Medial preoptic area: $F_{(2, 48)} = 17.42$, $p = 0.000$; Zona incerta: $F_{(2, 45)} = 6.21$, $p = 0.004$; Ventral tegmental area: $F_{(2, 45)} = 3.24$, $p = 0.046$). Animals in the 2 ppm group had fewer TH-IR neurons in the MPOA ($p < 0.001$), ZI ($p = 0.001$) and VTA ($p = 0.031$) than controls, and fewer TH-IR neurons than the 1 ppm group in the Arc ($p = 0.001$) and VTA ($p = 0.030$). The 1 ppm group possessed more TH-IR than controls in the Arc ($p < 0.001$) and fewer TH-IR in the MPOA ($p < 0.001$) (Figs. 4B and 5). We found a negative correlation between TH-IR neurons in the VTA and forced swimming test ($r = -0.939$, $p = 0.000$), and a positive correlation between TH-IRneurons in the VTA and novel object recognition ($r = 0.840$, $p = 0.000$).

4. Discussion

Exposure to different concentrations of FA in Kunming mice affects body mass, locomotor activity, depression- and anxiety-like behavior, and cognition in a similar way to other chemical stressors (Batis et al., 2010; Dallman et al., 2003; Makino et al., 2002; Seasholtz, 2000).

FA inhalation at 2 ppm markedly reduced body weight and this is consistent with previous work that found a reversible decrease in food and water consumption, body weight and body weight gain in rats at FA concentrations of 6.1 and 12.2 mg m^3-1,12.2 and 24.4 mg m^3-1, 6 and 12 ppm, respectively (Ozen et al., 2002, 2003; Songur et al., 2003; Zararsiz et al., 2006). These toxic effects may occur by central inhibition or inhibition of nucleic acid and protein synthesis (Ozen et al., 2003). However, body weight did not change following 1 ppm exposure in the current study and this is consistent with a previous study where FA exposure at 0.7 ppm for 1 week did not affect body weight (Sorg et al., 2001b). We conclude that FA exposure at 1 and 2 ppm affects body weight differently.

Mice exposed to 2 ppm FA showed less spontaneous locomotor activity (fewer total transitions and less distance) in the OFT which is consistent with a report that 2 ppm FA exposure for one week significantly reduced the spontaneous locomotor activity (SMLA) (Usanmaz et al., 2002), and increased time spent immobile during the FST, indicative of depression-like behavior. Previous studies have indeed observed chemical stressor-induced anxiety- and depression-like behavior in rodents (Batis et al., 2010), a typical subtype of depression is associated with a hyperactive HPA axis (Angst et al., 2002; Antonijevic, 2006), and these patterns are consistent with our results. However, mice exposed to 1 ppm FA did not show altered spontaneous motor activity which is consistent with one previous study (Sorg et al., 2001b). In addition, our data indicates that 1 ppm animals spent more time in the center of the OFT and more time in the open arms in the EPM than 2 ppm FA and control animals, displaying reduced levels of anxious behavior. Determining the in depth mechanisms underlying these alterations requires further investigation.

Mice in the 2 ppm groups displayed cognitive impairment during the NOR test, which was consistent with a study whereby inhalation toluene exposure impaired learning and memory in rats in a novel object test (Alejandra et al., 2012). Several studies showed that FA exposure can induce a deficit in spatial learning and memory (Malek et al., 2003b, 2002; Pitten et al., 2000; Lu et al., 2008), but the novel object recognition test measures cognitive capability (Lynch et al., 2011), and the symptoms of cognitive dysfunction are often accompanied by anxiety and depressive disorders (Fan et al., 2010; Engin and Treit, 2007). Mice in the 2 ppm FA group displayed increased depression-like behavior and was possibly accompanied with cognitive deficits.

Consistent with altered behavior in the 2 ppm animals, 2 ppm FA exposure reduced GR-IR neurons in the hippocampus in these animals since hippocampal GRs are important sites for negative feedback in the HPA axis. Thus, lower levels of hippocampal GRs reduced the sensitivity of feedback action of the HPA axis, and subsequently induced depression (Sapolsky et al., 1986; Webster et al., 2002). The involvement of GR downregulation in the pathophysiology of depression has been documented in clinical and

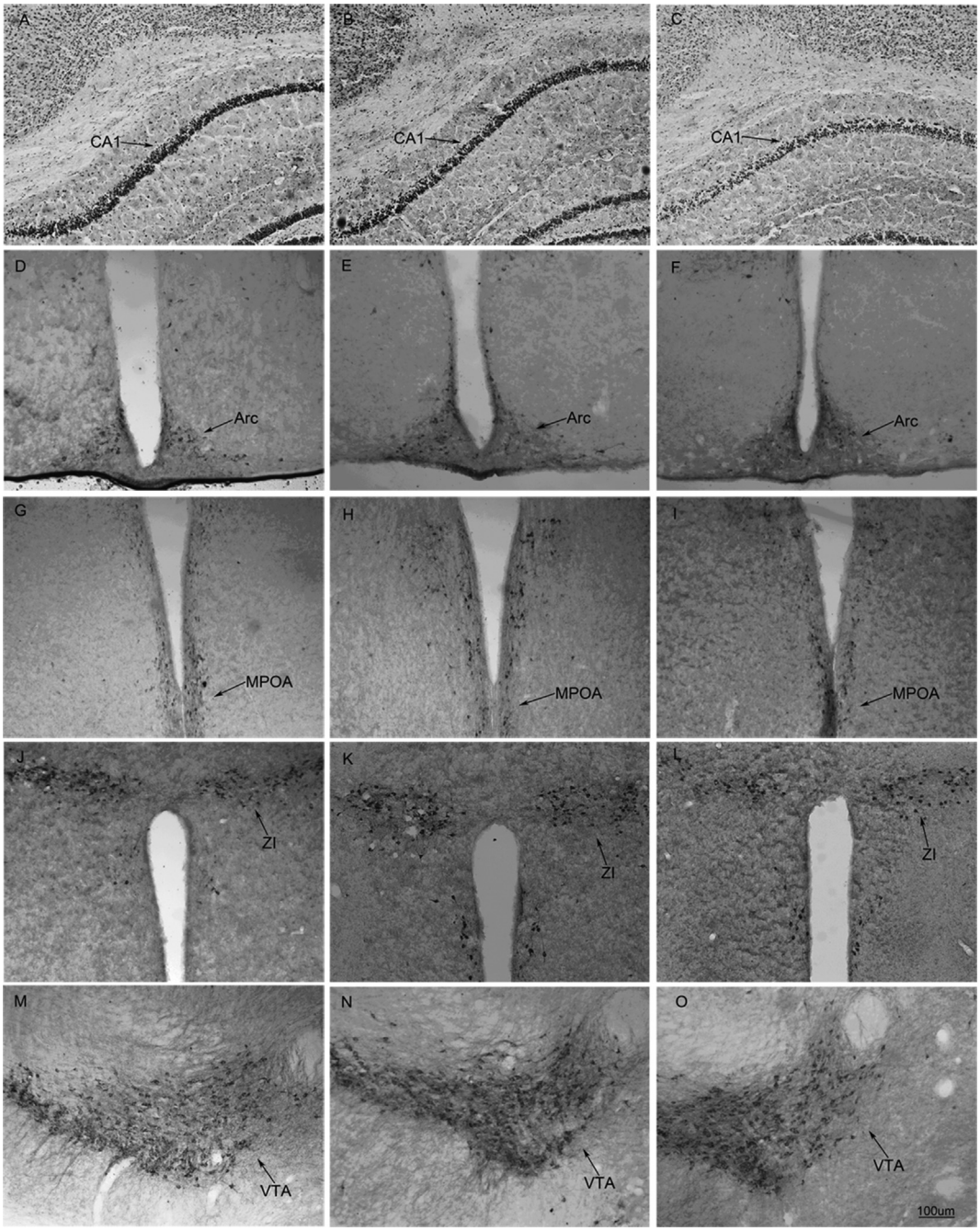

Fig. 5. GR-immunoreactive (GR-IR) neurons in the hippocampus (CA1) of mice (A: control; B: 1 ppm group; C: 2 ppm group). TH-ERs in the Arc (D, E and F), MPOA (G, H and I), ZI (J, K and L) and VTA (M, N and O) in control group; 1 ppm group and 2 ppm group. Bar = 100 μm.

preclinical studies (reviewed by Holsboer, 2000) and many studies suggest that external glucocorticoids exacerbate depression- and anxiety-like behaviors (Gonzalez-Perez et al., 2001; Hill et al., 2003; Kajiyama et al., 2010), and downregulate GR expression (Chao et al., 1998). Therefore, exposure to 2 ppm FA may be a chemical stress which leads to elevated cortisol (Sorg et al., 2001a), hippocampal neuronal loss and therefore GR loss (Sapolsky et al., 1986). This effect of chemical stress is supported by another study whereby chronic exposure to low levels of FA in rats caused an increase in the number of CRH-IR neurons in the hypothalamus (PVN) and ACTH-IR cells in the pituitary gland, with an increase in ACTH-mRNA expression in a dose-dependent manner (Sari et al., 2004). ACTH release can result in the release of corticosteroids from adrenal glands, subsequently, through mineralocorticoid and glucocorticoid receptors, exerting negative feedback on the hippocampus, pituitary and hypothalamus (Swaab et al., 2005). In addition, impaired glucocorticoid receptor function compromises cognitive and spatial capacity deficits (De Kloet et al., 1999). Mice in the 2 ppm FA group displayed increased depression-like behavior and fewer hippocampal GR-IR neurons and both factors contribute to cognitive deficits. However, 1 ppm had no effect on numbers of GR neurons in the hippocampus and did not increase depression, consistent with a previous study whereby FA exposure of 0.7 ppm for 1 week had no effect on basal HPA activity (Sorg et al., 2001a).

The further experiment in current study showed that inhalation FA exposure at 2 ppm significantly elevated the FA levels in brains. This result indicated that FA can easily passes through the blood–brain barrier, and eventually accumulate in human body

(Nazaroff and Singer, 2004). Previous studies have found that injection of formaldehyde at pathological levels (0.5 mM) directly into the hippocampus of rodents induces a deficit in spatial learning and memory in a Morris water maze test (Pitten et al., 2000; Malek et al., 2002, 2003b; Lu et al., 2008; Tong et al., 2015). Furthermore, growing evidence indicates that formaldehyde could play an important role in the induction of Alzheimer's disease (Tong et al., 2013, 2015; Qiang et al., 2014), because excess endogenous formaldehyde could induce memory loss in age-related memory-deteriorating diseases (Tong et al., 2011).Thus, here we suggested that FA in brains following inhalation FA exposure may affect novel object recognition in a more direct pathway in addition to chemical stress in the present study. However, whether FA in the hippocampus impairs novel object recognition via reduction of GR in the hippocampus remains unclear.

Dopamine is an immensely important central neurotransmitter and has a key role in a range of neurochemical and neurohormonal functions including cognition, sexual behavior, motor activity and emotional behavior (Gainetdinov et al., 1999; Rodgers et al., 1994; Zhuang et al., 1999; Zhou and Palmiter, 1995). Dopaminergic neuronal cell bodies originating in the substantia nigra (SN), hypothalamus, VTA, Arc and ZI project to various brain structures (Baskerville and Douglas, 2010). TH, as a rate limiting enzyme for DA synthesis, is an indicator of DA production. FA exposure at a concentration of 2 ppm reduced the level of TH-IR neurons in the MPOA, ZI and VTA, indicating that DA synthesis was reduced in the brain. Several studies have demonstrated that DA neurons in the VTA project to limbic regions including the nucleus accumbens, amygdala, hippocampus, and frontal cortex, which are closely associated with emotion and cognition (Herman et al., 1982; Thierry et al., 1976; Tidey and Miczek, 1996). Recent discoveries suggest that there may be distinct populations of VTA neurons that are preferentially activated by rewards or stress (Krishnan and Nestler, 2010; Veenema et al., 2007). Many studies have found that extracellular DA levels are negatively associated with levels of depression-like behavior (Tan et al., 2015). More lines of evidence also support that deficit of dopamineergic function in the limbic circuitry and striatum may possibly contribute to induction of depression (Tye et al., 2013; Park et al., 2005). In addition, DA can improve novel object recognition memory (Pezze et al., 2015; Rossato et al., 2013) and depletion of DA can possibly affects spatial learning and memory (Braun et al., 2015). We found a negative correlation between TH-IR neurons in the VTA and forced swimming test and a positive correlation between TH-IR neurons in the VTA and novel object recognition, consistent with these previous reports. Although the mechanism responsible for changes in behavior following FA exposure are not completely understood, alteration in the activity of TH may be a likely factor (Zigmond et al., 1989). Although we do not know the correlation between increased TH-IR neurons in the Arc and reduced anxiety in the 1 ppm FA group, we at least have evidence of increased DA synthesis in this brain region.

In this study we show that repeated exposure to FA at 2 ppm can affect locomotor activity, depression-like behavior and novel object recognition. These alterations in behavior may be associated with hippocampus GR and central nervous system TH. However, this study provides very limited evidence for the effect of inhaled FA on reduced anxiety levels and nervous toxicology in 1 ppm mice. Further, evidence is lacking for a relationship between reduced anxiety and dopaminergic activity in the 1 ppm animals.

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